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ETIOLOGY OF EXPERIMENTAL SHOCK

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l. Passive Transfer of a Lethal Factor in the Blood of Rabbits Shocked by Occlusion of the Superior Mesenteric Artery (SMA). Samples of Blood were collected from the portal vein of rabbits (donors) after shock had been induced by SMA occlusion and release. The samples were injected into test rabbits (recipients) whose defenses had been weakened by an episode of sublethal, hemorrhagic hypotension. About half of the tested recipients died, whereas normal portal blood (collected from shamoperated donors) failed to kill any of a group of similarly prepared test-recipients. The incidence of passive transfer of lethality in these experiments closely matched the incidence of lethality resulting from MA-occlusion shock itself.

- 2. Evidence Against Bacterial Endotoxin as the Lethal Factor in Blood ving SMA-Occlusion Shock. Although whole blood from SMA-occlusion-shocked donors proved lethal to prepared recipients, plasma fractions obtained from these same lethal blood samples failed to kill any prepared recipients. When donor rabbits were first pretreated with non-absorbable antibiotics per os, the number of actively reproducing bacteria in their intestinal fluids was reduced to less than 0.1% of normal. Nevertheless, there was no consistent reduction in lethality of portal blood samples collected from these donors. Furthermore, when shock portal blood samples were tested for the presence of bacterial endotoxin by a sensitive dermal epinephrine test (epinephrine-accelerated dermal hemorrhagic necrosis), positive reactions were obtained almost exclusively with non-lethal blood samples.
  - 3. A Biological Effect of Elevated Serotonin Levels in SMA Occlusion Shock Blood. Evidence was obtained indicating that the production of dermal epinephrine reactions by non-lethal, shock-blood samples is due to elevated levels of serotonin in such blood. SMA-occlusion shock was shown to cause elevations in 5-HT content of intestinal tissues and of portal blood. Intravenous injection of doses of 5-HT (equal to the 5-HT content of infused samples of shock blood) was capable, by itself, of producing epinephrine reactions in prepared recipients. In addition, the blood of Neomycin-pretreated animals was observed to produce the most severe epinephrine reactions, and this correlated well with the fact that Neomycin-pretreated rabbits possessed the highest concentration of intestinal 5-HT. It seems, therefore, that the production of dermal epinephrine reactions by blood from SMA-occlusion shocked rabbits was due to elevated levels of ASSHT in this blood, and not to the presence of bacterial endotoxin.

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#### PROGRESS REPORT

May 1, 1960 - April 30, 1961

#### STUDIES ON THE ETIOLOGY OF EXPERIMENTAL SHOCK

# I. Pathogenesis of Experimental Shock

# II. Detoxifying Function of the Reticuloendothelial System

This portion of our research program on experimental shock, begun in September 1958, has diverged along two major avenues over the past year. First, we have continued to investigate pathogenetic mechanisms in shock in animals; and second, we have broadened our study of the role of iron compounds in the detoxification mechanisms of the reticuloendothelial system (RES). The second part of this program developed from an earlier inquiry into possible interrelationships between deranged iron metabolism and bacterial endotoxins in shocked animals. The results obtained in our earlier studies in both of these areas have been summarized in progress reports covering the years 1958-1960 and were also reported in publications 1 - 5, cited below. The present report summarizes studies completed over the past year and, in addition, briefly describes work in progress.

# I. The Pathogenesis of Experimental Shock

# A. Work Completed

It has been reported that rabbits and dogs, whose resistance has been lowered by a short episode of hemorrhagic shock (hemorrhage-prepared animals), survive when reinfused with their own or other normal blood but die when given blood from animals in irreversible hemorrhagic shock, tourniquet shock, or shock induced by peritonitis. On the basis of these findings, Fine and co-workers have postulated the presence of a shock toxin in the blood of the irreversibly shocked animal. Further investigation led them to identify this lethal factor as an endotoxin, probably derived from the bacterial flora of the intestines. Among the lines of evidence supporting this identification are the findings: (a) that the lethal properties of irreversible shock blood are recoverable from the lipopoly-saccharide fraction of the plasma, and that (b) passive transfer of lethality is frequently prevented when the donor animal has been effect-ively pretreated with non-absorbable antibiotics, per os.

Recent tissue studies failed to provide morphological evidence for the presence of circulating bacterial endotoxin in experimental hemorrhagic shock. Obviously, the nature of the toxic material passively transferred by shock blood is still open to question. We have explored the matter further, using another form of experimental shock. Since the intestines have been implicated as the chief source of the shock toxin in the studies of Fine, Lillehei and others, shock was produced in our experiments by ligation of the major artery supplying blood to that organ and an attempt

was made to demonstrate a toxic principle in hepatic portal blood, using Fine's passive transfer technique. We were further interested in testing these portal blood samples for endotoxin-activity, since under the conditions of the experiment, bacterial endotoxin should have been present if, in fact, endotoxemia is a common toxic modality in different forms of experimental shock.

Portal blood was collected at intervals from donor rabbits following shock induced by occlusion and release of the superior mesenteric artery (SMA -shock). Infusion of this blood into sublethally hemorrhaged rabbits caused the death of half of the tested animals. This mortality incidence closely matched the per cent mortality in rabbits shocked by SMAO-occlusion alone. However, infusion of SMAO-shock plasma into hemorrhage-prepared rabbits did not result in the death of the recipients, even though the original whole blood had proven to be lethal. Moreover, when donor animals were pretreated with a non-absorbable antibiotic, per os, the number of actively reproducing intestinal bacterial were reduced to less than 0.1% of normal, despite the fact that the blood of these animals upon passive transfer was consistently lethal. Furthermore, when SMAO-shock portal blood was tested for the presence of bacterial endotoxin by the sensitive dermal epinephrine reaction, some blood samples demonstrated lesion-provoking activity, but there was no correlation between this activity and the lethal properties of the blood samples. On the other hand, a positive correlation was demonstrated between the lesion-provoking activity of portal blood and the serotonin content of intestinal tissues of rabbits shocked by SMA ligation. In addition, small amounts of serotonin were shown to be capable of provoking dermal epinephrine reactions in rabbits, under the same conditions used to test the lesion-provoking activity of portal blood. concluded that: (a) A toxic factor(s) is present in the portal blood of SMAO-shocked rabbits; (b) that this factor(s) is not likely to be a bacterial endotoxin; and (c) that the occasional provocation of a dermal epinephrine reaction by portal blood from SMAO-shocked rabbits, a property heretofore exclusively attributed to the presence of endotoxin in shock blood, can be entirely explained on the basis of elevated levels of serotonin in this blood.

#### B. Work in Progress

We plan to continue our study of the passive transfer of lethality in shock blood by investigating: (1) The appearance of this toxic factor(s) in shock blood with respect to the time of onset of irreversibility, (2) The nature of the lethal material, and (3) The alterations which develop in animals subjected to a sublethal hemorrhage (prepared animals) which are critical to the enhanced susceptibility to shock toxins.

To facilitate these studies, we are currently developing a technique for rapid, controlled hemorrhage of mice, in order to have available large numbers of hemorrhage-prepared animals as recipients for passive transfer studies. This should permit the application of statistical methods in the experiments just outlined. Removal of a quantity of blood equal to 3.0 or 3.2% of the body weight of mice is achieved by bleeding

from the retro-orbital plexus. The animals become rapidly debilitated and about 10% die within the first half-hour after bleeding. The remainder are visibly ill for at least two hours.

The effect of injecting normal blood and shock blood obtained from donor animals (rabbits and dogs) into mice prepared in this way will be examined next.

# III. Detoxifying Function of the Reticuloendothelial System

#### A. Work Completed

It has been known for many years that iron metabolism becomes altered in a variety of infections and toxemic states. In these conditions, the levels of serum protein-bound iron and ferritin of liver and spleen decrease, while the hemosiderin content of spleen and other reticuloendothelial (RE) organs is markedly increased. These disturbances in iron metabolism during infection reflect a shift from normal pathways -- iron absorption, storage, and utilization in hemoglobin formation -- to pathways leading to a rapid increase in RE intracellular hemosiderin. The data suggest that hemosiderin-iron may be involved in the detoxifying activities of phagocytic cells. It has, in fact, been shown that hemosiderin and low molar concentrations of inorganic iron salts con inactivate, in vitro, toxic substances of bacterial and tissue origin. also been reported that, under certain conditions, parenteral administration of colloidal or inorganic iron enhances the resistance of guinea pigs to the lethal effects of diphtheria toxin and of mice to certain clostridial toxins. A recent publication from this laboratory confirmed and extended the toxin-inactivating action of inorganic iron, in vitro. We have recently undertaken an investigation of iron detoxifying activity in vivo.

A study was made of the ability of colloidal iron (Proferrin) to protect mice against a minimum lethal dose of Cl. perfringens alpha toxin, after incorporation of the iron in the RES of these animals. The protection apparently was the result of a general effect of colloid treatment. It was therefore unnecessary to postulate a direct detoxifying effect of the intracellular RE-iron. Moreover, an equivalent degree of protection was afforded to mice after pretreatment with an iron-poor colloid (heat denatured albumin), which exerted very little effect on phagocytic index or iron metabolism. In addition, colloidal iron treatment did not protect mice against the lethal effects of bacterial endotoxin (S. enteritidis); but, exacerbated the lethality of this material to a degree predictable from the endotoxin-lethality-enhancing behavior of other colloids.

Studies were also made of the effects of RE cell preparations upon the rate of combination of Cl. perfringens alpha toxin and iron, in vitro. Some, but not all, homogenates prepared from normal mouse spleen were capable of moderately accelerating the toxin-iron reaction. Spleen homogenates prepared from stress-adapted rats had no effect on the rate of the iron-toxin reaction. Mouse peritoneal macrophages, lysed by

ultrasonication, likewise had no effect upon this reaction. It was therefore concluded that RE tissues of the mouse and rat do not contain a factor, preservable in crude homogenates, which is capable of accelerating the combination of inorganic iron and Cl. perfringens alpha toxin, in vitro.

### B. Work in Progress

The experiments just described involved reactions between inorganic iron and bacterial toxins. The failure of spleen homogenate to accelerate this reaction does not rule out the possibility that similar spleen homogenates contain factors which would be capable of accelerating the interaction of toxins and organically bound iron, as found in spleen RE cells. Therefore, it seems desirable to repeat the homogenate experiments using hemosiderin as an iron source, instead of inorganic salts of this metal. We have recently obtained a purified preparation of hemosiderin from horse spleen and have begun these studies. The normal rate of inactivation of Cl. perfringens alpha toxin by horse spleen hemosiderin has been determined and we are now studying the effect of the addition of homogenates of horse spleen upon the rate of this reaction, as measured by toxicity tests in mice. In addition, we are comparing the rates of release of ionic iron from hemosiderin incubated alone and in the presence of horse spleen homogenate.

B.W. Zweifach and A. Janoff

#### **PUBLICATIONS**

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